REMARKS

Claims 1-12, with claims 1 and 2 being the independent claims, are pending and presented for examination in connection with the subject application.

Applicants have hereinabove amended independent claims 1 and 2 to place the claims in better form for examination, without narrowing the scope of the claimed invention.

Applicants maintain that no new matter is presented by this amendment. Accordingly, Applicants respectfully request that this Amendment be entered.

Rejection under 35 U.S.C. §112, second paragraph

In Section 3 of the July 15, 2002 Office Action, claims 1-12 were rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

The Examiner stated that the "dendritic cell culture medium" of claim 7 has not been defined in the specification. The Examiner further stated that the third paragraph of page 8 of the specification discloses "a" (indicating others exist) dendritic cell culture medium. The Examiner also stated that said medium consists of just three components which are disclosed only as "e.g." (indicating that the disclosed concentrations are only for example only and not defining). The Examiner further stated that the claims recite a specific method, i.e., "reproducibly generating dendritic cells" employing a specific composition, i.e., "dendritic cell culture medium". The Examiner stated that said composition has not been defined, rendering the claims vague and indefinite.

The Examiner stated that the recitation of "incubating for a predetermined time period tissue culture" in claims 1, 2 and 8 comprises an ungrammatical phrase of unclear meaning. The Examiner further stated that the claims, as such, are considered vague and indefinite.

Applicants have hereinabove amended claims 1 and 2 to place the claims in better form for examination, without narrowing the scope of the claimed invention. Applicants respectfully submit that the amended claims clearly recite the subject matter Applicants regard to be the invention.

Applicants maintain that the term "dendritic cell culture medium" in claim 7 is adequately described in the application. For example, one embodiment is described at page 8, lines 9-21 and at page 9, lines 34-36 of the application as follows, respectively:

> "Another embodiment will now be described with reference to FIGS. 6A through 6D.
>
> A dendritic cell culture medium is prepared (step 701) by combining AIM V media (e.g., BB-MF 2557, Life Technologies, Grand Island, NY), rh-IL-4 (e.g., 1000 U/ml, GLP grade, Sigma Aldrich, St. Louis, MO) and rh-GM-CSF (e.g., 25 ng/ml, Therapeutic grade, Immunex, Seattle, WA). ..."

> "Next, the dendritic cell culture medium (prepared in step 701) is transferred via a sterile process to the tissue culture bags (step 710). ..."

As another example, the application states at page 7, lines 5-9 as follows:

> "After removal of nonadherent cells, 100 ml of AIM-V media containing rh-GM-CSF (e.g., 25 ng/ml, Sargramostim, Immunex, Seattle, WA) and rh IL-4 (e.g., 1000U/ml, Sigma, St. Louis, MO) is introduced into the tissue culture bags (step 408). ..."

In addition, the application points out (at page 1, line 28 through page 2, line 2) that dendritic cell systems may be "initiated from the adherent fraction of peripheral blood mononuclear cells, selected using open polystyrene flasks, followed by washing and then culture in serum-free medium containing GM-CSF and IL-4 or IL-7 (as well as other maturational cytokines) {G. Schuler et al., *Dendritic cells as adjuvants for immune-mediated resistance to tumors*, 186 J. Exp. Med. 1183-87 (1997); M. Di Nicola et al., *Human dendritic cells: natural adjuvants in antitumor immunotherapy*, 4 Cytokines Cell Mol. Ther. 265-73 (1998)}."

Therefore, Applicants maintain that the term "dendritic cell culture medium" in claim 7 would be understood by one of ordinary skill in the art in view of the specification of the subject application, to include culturing precursor cells in the presence of cytokines, notably granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 or IL-7 (as well as other maturational cytokines). Additional maturation factors of course may be added to the dendritic cell culture medium, such as recombinant tumor necrosis factor alpha (TNF α) [for example, available from R&D Systems, Minneapolis, MN], recombinant soluble CD40L trimeric fusion protein (for example, available from Immunex Corporation), interferon gamma (IFN γ) [for example, available from Actimmune, Intermune Pharmaceuticals, Palo Alto, CA].

Applicants maintain that recitation of "incubating the container for a second predetermined time period" in claim 8 complies with the rules of grammar and no amendment of the claim is needed.

Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1-12 under 35

U.S.C. §112, second paragraph.

Rejection under 35 U.S.C. §112, first paragraph

In Section 5 of the July 15, 2002 Office Action, claims 1-12 were rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Examiner stated that the specification disclosure is insufficient to enable one skilled in the art to practice the invention as claimed without an undue amount of experimentation. The Examiner further stated that undue experimentation must be considered in light of factors including: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill in the art, the level of predictability of the art, the amount of direction provided by the inventor, the existence of working examples, and the quantity of experimentation needed to make or use the invention.

The Examiner also stated that the instant invention is drawn to a method of "reproducibly generating dendritic cells" comprising a method of cell culture. The Examiner further stated that in vitro generation of dendritic cells was well known in the art at the time of the invention of the instant claims. The Examiner stated that performing the steps of the instant claims would not necessarily result in a product consisting of said cells given the breadth of the claims, i.e., the lack of specific limitations. The Examiner further stated that the method of the instant claims must be considered highly unpredictable and requiring of undue experimentation.

The Examiner stated that step (a) recites the loading of "blood

mononuclear cells" into a cell culture container. The Examiner further stated that the specification discloses that it is a "requirement" that the method of the instant claims begin with monocytes and monocyte precursors separated from lymphocytes. The Examiner also stated that the method of the instant claims would be highly unpredictable given that the recitation of "blood mononuclear cells" would include lymphocytes in the starting material.

The Examiner stated that step (b) recites "incubating for a predetermined time period". The Examiner further stated that a specific time period is not disclosed. The Examiner also stated that step (c) requires "separating adherent and nonadherent cells", and the incubation time period is a critical feature of the claimed method. U.S. Patent No. 5,851,756 teaches that, depending on the length of incubation, dendritic cells are either nonadherent, loosely adherent, or adherent. The Examiner stated that the recitation of "incubating for a predetermined time period" alone (without the recitation of any specific time period) renders the method of the instant claims highly unpredictable as it would be unknown whether the method encompassed the further culture (in step (f)) of the adherent or the nonadherent cells.

The Examiner stated that, regarding step (c) in claim 1 and step (d) in claim 2, and the recitation of "separating nonadherent cells and cells adhered to the beads", claims 1 and 2 fail to indicate which group of cells is used to generate dendritic cells. The Examiner further stated that the method of the claims must again be considered highly unpredictable because it would appear that the method intends the further culture of one group of cells and the discarding of the other group, but the claims fail to indicate which group is saved and which group is

discarded.

The Examiner stated that step (f) recites "incubating the container for a second predetermined time period". The Examiner further stated that a specific time period is not disclosed. The Examiner also stated that, as the "predetermined time period" is not disclosed, the method must again be considered highly unpredictable as too short a period (e.g., hours) would not result in the generation of dendritic cells, while too long a period (e.g., months) would result in a dead culture. The Examiner further stated that U.S. Patent No. 5,851,756 teaches an optimal incubation period of several days.

The Examiner stated that, regarding the incubation of the blood mononuclear cells of the instant claims, the generation of dendritic cells from said blood mononuclear cells would require the inclusion of specific reagents in the incubation, at minimum GM-CSF (in all dendritic cell cultures) and TGF-β (in human dendritic cell cultures).

The Examiner stated that, regarding the method of claim 12, wherein a ratio has a value sufficient to hold enough media for incubation, the specification fails to disclose what values would be considered sufficient. The Examiner further stated that, as recited, the value would encompass a surface area ranging from that of the container alone (no microcarrier beads), to a container packed full of microcarrier beads. The Examiner also stated that it is unclear, however, if either of said values would allow for the reproducible generation of dendritic cells. The Examiner further stated that the method of the instant claim must be considered highly unpredictable and requiring of undue experimentation.

The Examiner stated that it is here established that simply performing the method of the instant claims, as broadly claimed, would not result in the "reproducible generation of dendritic cells". The Examiner further stated that the method of the instant claims must be considered highly unpredictable and requiring of undue experimentation. The Examiner also stated that said undue experimentation in turn indicates that the specification fails to adequately disclose how to make and use the invention of the instant claims.

The Examiner stated that the specification discloses no actual data demonstrating the method of the instant claims, i.e., no working examples. The Examiner further stated that while working examples are not required, given the unpredictable nature of the methods encompassed by the breadth of the instant claims, some demonstration that the method would function in its breadth is required. The Examiner also stated that said demonstration would in particular address the internal contradictions, e.g., the disclosure that the method must begin with monocytes or monocyte precursors while the claims recite the use of blood mononuclear cells, and said demonstration would additionally address the lack of any recitation of required culture conditions, e.g., the generation of dendritic cells from blood mononuclear cells without culturing under specific conditions, e.g., in GM-CSF. The Examiner further stated that absent said demonstration, the method of the instant claims must be considered to require undue experimentation.

The Examiner stated that In re Wands, 858 F. 2d at 737, 8 USPQ2d at 1404 (Fed. Cir. 1988) indicates that the more unpredictable an area is, the more specific enablement is necessary in order to satisfy the statute. The Examiner further stated that in view of the quantity of experimentation necessary, the lack of working

examples, the unpredictability of the art, and the breadth of the claims, it would take undue trials and errors to practice the claimed invention.

Applicants have hereinabove amended claims 1 and 2 to place the claims in better form for examination, without narrowing the scope of the claimed invention.

Applicants maintain that the claimed invention as recited in the amended claims is fully enabled by the specification and the specification reasonably conveys to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

The Office Action states that page 5, paragraph 4 of the application requires that the method of the instant claims begin with monocytes and monocyte precursors separated from lymphocytes.

Applicants respectfully disagree. Page 5, lines 19-25 of the application states as follows:

> "One requirement of blood mononuclear cell (MNC) products suitable for DC culture is collection of a maximum number of monocytes and monocyte precursors with a minimum number of red blood cells, lymphocytes and platelets. This may be accomplished by pheresing donors on an apheresis system (e.g., Spectra, COBE BCT, Lakewood, CO) using a mononuclear cell program."

The specification further states (at page 9, lines 24-31) as follows, regarding one embodiment:

> "The tissue culture bags then are incubated (step 708) ... At the midpoint, the bag is flipped from one side to the other. After four hours, the tissue culture is washed three times with AIM-V to remove nonadherent lymphocytes, platelets, grans, RBC, etc. (step 709). The wash includes transfer of the AIM-V media and expressing off the

supernatant while leaving the beads in the bag."

Accordingly, Applicants maintain that it is clear from a reading of the application that loading mononuclear cells into a cell culture container, as provided by the claimed invention, does not require monocytes and monocyte precursors entirely separated from lymphocytes. Indeed, separation of lymphocytes from the adherent cells is a result of applying the claimed method.

The Office Action states that the claims refer to incubating for a predetermined period of time and incubating for a second period of time, and that the specification does not provide specific periods of time.

Applicants maintain that, to the contrary, the specification provides adequate guidance regarding exemplary periods of incubation. For example, the application states at page 9, line 24 through page 10, line 5 as follows:

> "The tissue culture bags then are incubated (step 708), for example, in a humidified 37°C, 5% CO_2 atmosphere for **approximately four hours**. At the midpoint, the bag is flipped from one side to the other. After four hours, the tissue culture is washed three times with AIM-V to remove nonadherent lymphocytes, platelets, grans, RBC, etc. (step 709). The wash includes transfer of the AIM-V media and expressing off the supernatant while leaving the beads in the bag.
>
> Next, the dendritic cell culture medium (prepared in step 701) is transferred via a sterile process to the tissue culture bags (step 710). The bags are incubated again, for example, in a humidified dedicated 37°C 5% CO_2 incubator (step 711) **for 5 to 7 days**. At day 4, samples of cell suspension are removed for quality control (step 712). On day 7, the tissue culture bags are moved from the incubator to a biological safety cabinet (step 713). ..."

The exemplary periods of time for incubation may be adapted of course according to, for example, container volume, culture

volume, etc.

In addition, the application also describes use of reagents, at page 7, lines 5-11 as follows:

> "After removal of nonadherent cells, 100 ml of AIM-V media containing rh-GM-CSF (e.g., 25 ng/ml, Sargramostim, Immunex, Seattle, WA) and rh IL-4 (e.g., 1000U/ml, Sigma, St. Louis, MO) is introduced into the tissue culture bags (step 408). The bags may be placed into a dedicated, Hepa-filtered, humidified 37°C 5% CO_2 incubator (step 409) for 7 days. ..."

Accordingly, Applicants maintain that the specification provides adequate guidance regarding periods of incubation.

The Office Action states that performing the steps recited in the claims of the subject application would not necessarily result in the product recited in the preamble of the claim. The Office Action further states that the application does not provide a working example. The Office Action also states that, while claims 1 and 2 recite "separating nonadherent cells and cells adhered to the beads", the claims fail to indicate which group of cells is used to generate dendritic cells.

Applicants respectfully point out that the claims of a patent application define the boundaries of the subject matter which the applicant claims as applicant's invention, but the claims need not, and indeed usually cannot, set forth all of the details of the subject matter. It is well established in Federal Circuit case law that use of the term "comprising" in a claim, such as in the subject claims, allows the recitation of elements (or steps) in the claim to be open to additional elements (or steps) not specifically mentioned. Further, 35 U.S.C. 112 requires the specification, and not the claims, to provide an enabling description.

In addition, the application provides a number of exemplary, enabling embodiments. For example, a working embodiment, corresponding to FIGS. 6A-6D, is described at page 8, line 9 through page 12, line 19.

It is also well established that one looks to the specification for guidance for practicing the claimed invention, and for a written description which indicates that the inventors had possession of the claimed invention at the time the application was filed.

The subject application states at page 9, lines 24-32 as follows, regarding one exemplary embodiment:

> "The tissue culture bags then are incubated (step 708), for example, in a humidified 37°C, 5% CO_2 atmosphere for approximately four hours. At the midpoint, the bag is flipped from one side to the other. After four hours, the tissue culture is washed three times with AIM-V to remove nonadherent lymphocytes, platelets, grans, RBC, etc. (step 709). The wash includes transfer of the AIM-V media and expressing off the supernatant while leaving the beads in the bag."

In addition, the application provides the following guidance at page 12, line 27 through page 13, line 12 (in relevant part:

> "... The bags alone, however, do not provide an ideal surface for the attachment of DC precursor cells (monocytes). The introduction of selected polystyrene beads into the bags provides a surface that the monocytes easily adhere to. Once the monocytes have matured into DC, their adherence to the polystyrene surface, provided by the beads, is significantly reduced. At the end of the culture period, DCs no longer adhere to the beads and are harvested in the supernatant.
>
> ... Since the monocytes adhered to the beads settle with the beads and thereby separate from the undesired cells (e.g., lymphocytes, platelets, etc.) which are removed by expressing off the supernatant."

Applicants maintain that the application provides sufficient guidance for practicing the claimed invention.
According to the Office Action, the application does not provide sufficient guidance for determining a ratio which allows the container to hold enough media for the predetermined time period of incubation.

The application provides guidance on, for example, page 13 for selecting appropriate microcarrier beads and an appropriate cell culture container. For example, page 13, lines 1-4 states as follows:

> "The beads are selected based, in part, on their size. Since more surface area is desirable, smaller beads in a larger quantity is preferred to larger beads in a smaller quantity."

In addition, the application states as follows at page 13, lines 31-35:

> "... it is important to maintain a ratio of (beads and container) surface area to container volume that allows the container to hold enough media to support the culture period, so that culture only needs to be fed once, rather than repeatedly."

According to one exemplary embodiment described in the application, a 180 cm^2 cell culture container and microcarrier beads having a diameter of 250 microns are used

Applicants maintain that the application provides adequate guidance for determining suitable sizes for a cell culture container and microcarrier beads, respectively, without undue experimentation.

Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1-12 under 35

U.S.C. §112, first paragraph.

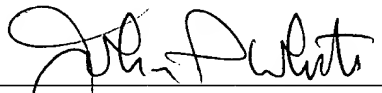
In view of the amendments to the claims and remarks hereinabove, Applicants maintain that claims 1-12 are now in condition for allowance. Accordingly, Applicants earnestly solicit the allowance of claims 1-12.

If a telephone interview would be of assistance in advancing prosecution of the subject application, Applicants' undersigned attorney invites the Examiner to telephone him at the telephone number provided below.

If a petition for an extension of time is required to make this response timely, this paper should be considered to be such a petition, and the Commissioner is authorized to charge the requisite fees to our Deposit Account No. 03-3125.

No fee is deemed necessary in connection with the filing of this Amendment. However, if any additional fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,



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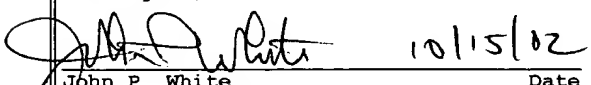

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